Hepatitis B

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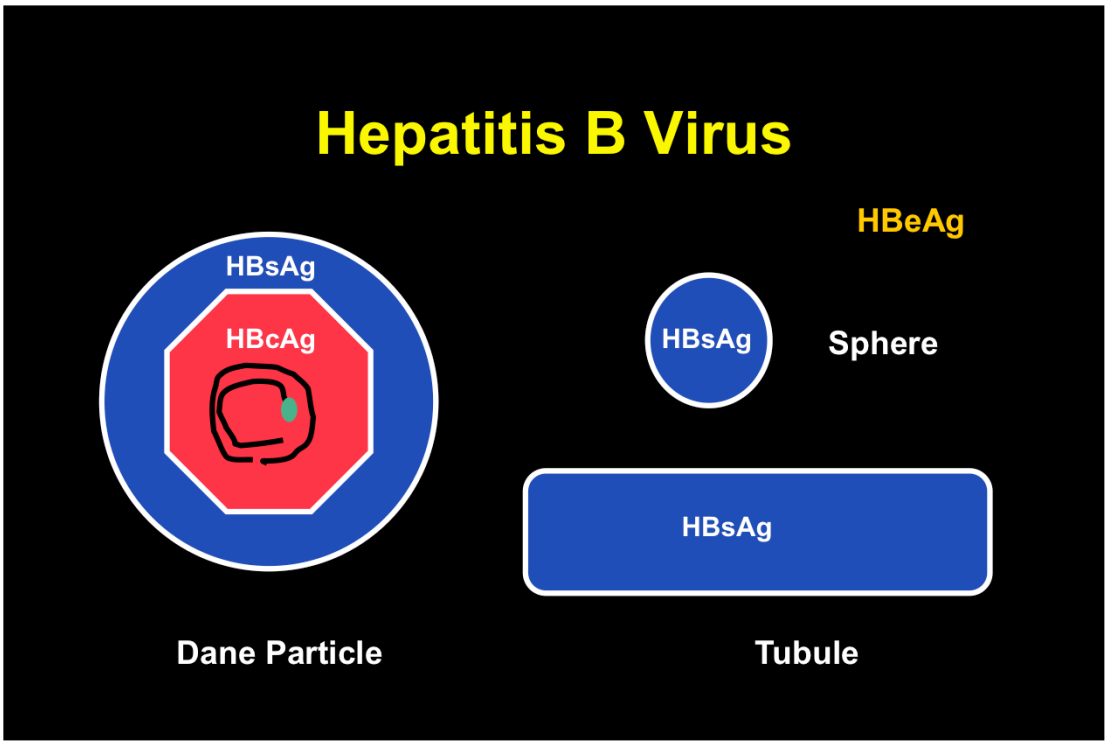
Hepatitis B

- A challenging and ancient disease
- Due to a small DNA virus
- Causes both acute and chronic liver disease
- The single major cause of cirrhosis and liver cancer worldwide
- Yet, preventable with HBV vaccine
- And now, treatable with antiviral agents

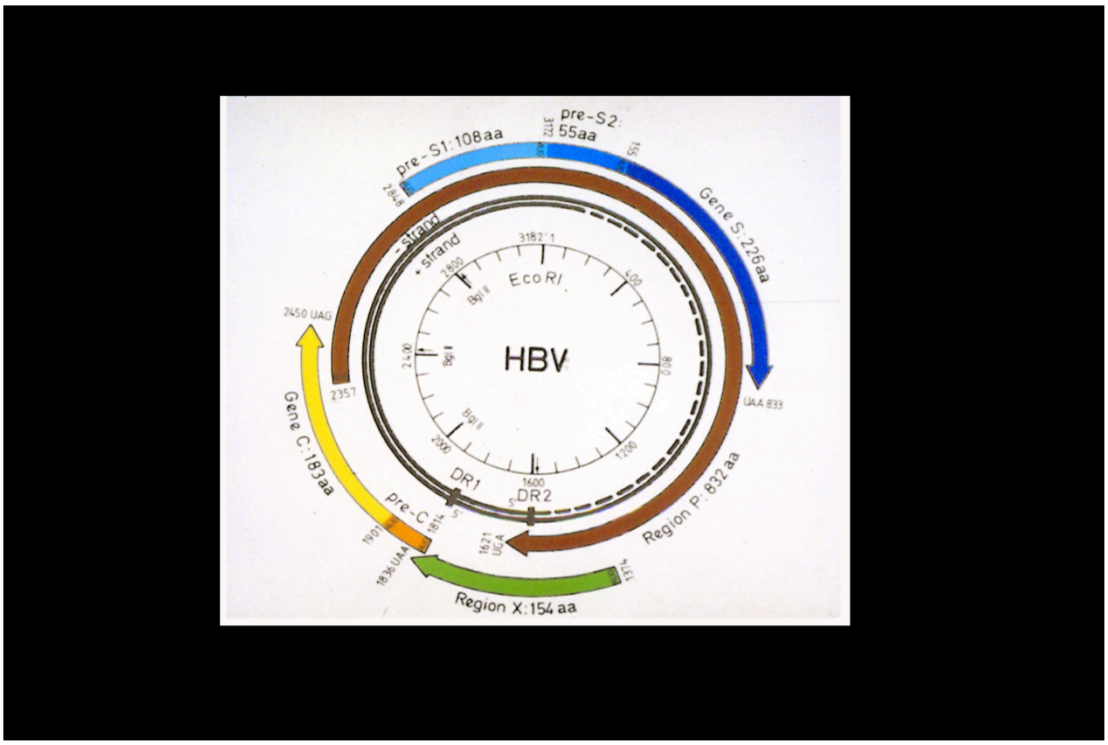
Hepatitis B Virus

- HBV, small double stranded DNA virus
- Hepadnaviridae
- Infection restricted to humans and higher apes
- High levels in blood (10^2 to 10^{10} copies/ml)
- Causes both acute and chronic hepatitis
- Parenteral, sexual and maternal-infant spread
- Marked geographic variation in incidence
- Common in Asia & Africa, uncommon in the United States and Western Europe

The first member of the viral genus hepadnaviridae; others include the Woodchuck, Duck and Heron hepatitis viruses. Levels in the blood are higher than that achieved by any other human virus in non-immunosuppressed persons.



A cartoon displaying the three forms of HBsAg found in serum. Most plentiful are small incomplete spheres made up of (small) HBsAg alone, variously sized tubules of HBsAg are also present. The complete virion known as the Dane particle (for Dr. David Dane) has an outer envelope of HBsAg and an inner nucleocapisid of HBcAg (core) within which is a circular, double stranded molecule of HBV DNA to which is attached (in green) an enzyme: HBV DNA polymerase. Also found in serum is HBeAg, the third HBV antigen, which is non-particular, but rather a small molecular weight protein (19 K dalton) whose significance is becoming clear only now.

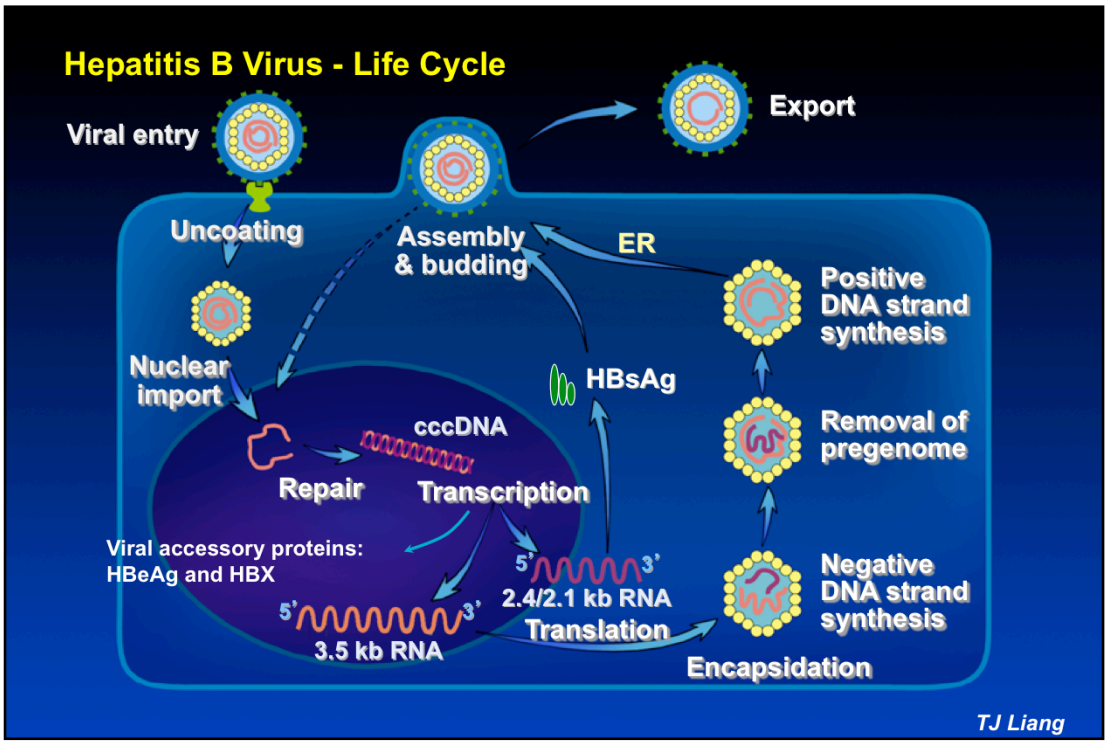


A cartoon of the HBV genome, showing the double stranded molecule (in black) with a number system based on an EcoR1 restriction site. The negative strand is nicked (not shown very well but between DR1 and DR2) and the positive strand is incomplete (to varying extents as shown by the dashed line). The genome has 4 major open reading frames that represent HBsAg (S: in blue), HBcAg (C in yellow), DNA polymerase (P in brown), and HBxAg (X in green; a protein of unclear significance). The S gene has three start signals and thus can produce three forms of HBsAg: small, medium and large – the large S (pre-S1) having the attachment or receptor site for the antigen and the small S being the major form found in the small spheres in blood. The C gene also has two starts sites; synthesis starting at the first includes the pre-C region and results in production of the secreted protein HBeAg; synthesis starting at the second start codon results in HBcAg. Thus HBeAg and HBcAg share much of the same amino acid sequence, but are different in tertiary structure. The HBV genome is one of the smallest DNA genomes in virology and is amazingly “compact” with every base pair being used twice! And synthesizing different proteins on the small DNA molecule because the start sites are all “out of frame”. No junk DNA here! Even regulatory sequences are embedded in the genome, in regions that are also protein coding.

Hepatitis B Viral Genome

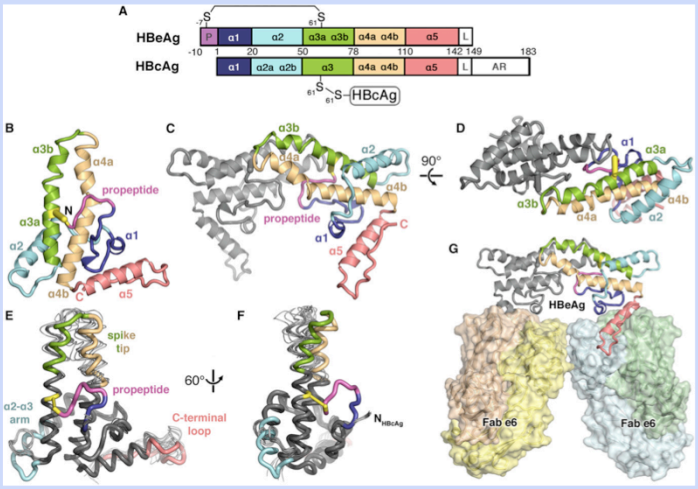
- Circular, partially doubled-stranded DNA
- Four open reading frames
  - HBsAg (pre-S1, pre-S2 and S)
  - HBcAg (pre-core & core)
  - Polymerase (multifunctional)
  - HBxAg (transactivating factor)
- Replicates largely in liver
- Through RNA intermediate and reverse transcription

To make things even more complex, HBV replicates not by DNA to DNA synthesis, but first through RNA. The polymerase gene of HBV is multifunction, operating as both a RNA dependent (reverse transcriptase) and a DNA dependent polymerase. Only the Hepadnaviruses have this mode of replication. How did such a complex and compact, efficient virus evolve? Obviously, it is "older than man" having probably jumped from avian or rodent hepadnaviruses sometime in pre-history.



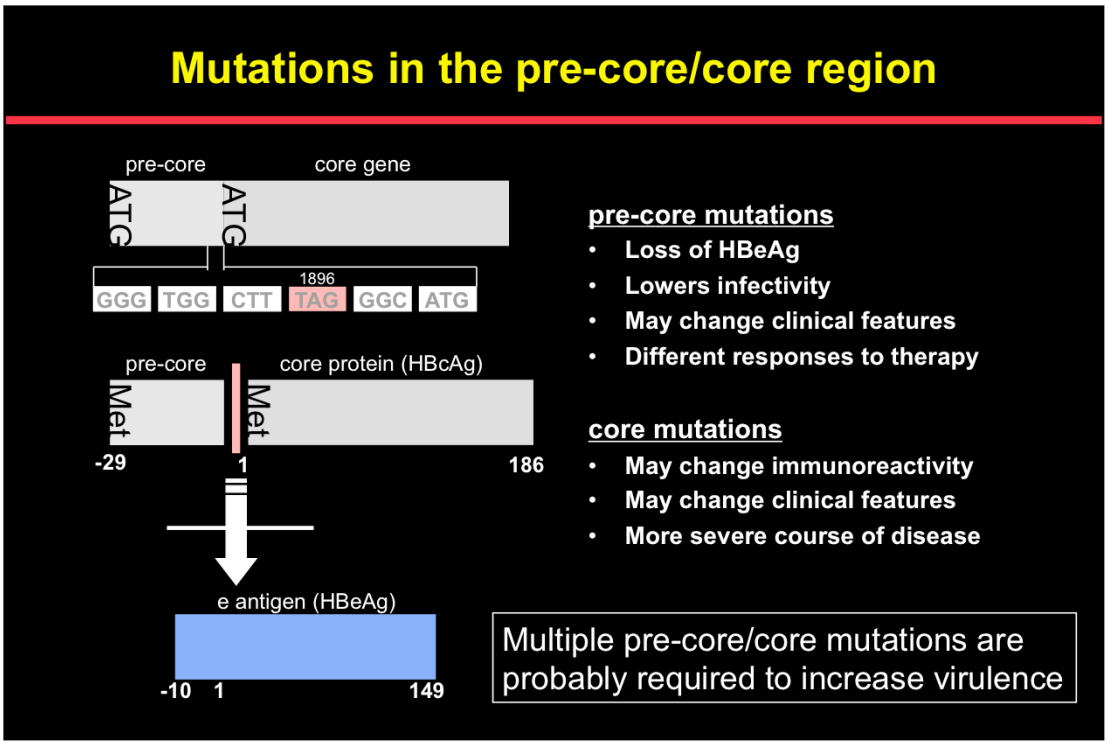
The complex life-cycle of HBV, replication occurring largely in hepatocytes. The virus enters the cell via cell attachment and receptors and the HBsAg is removed in the cytoplasm, causing the nucleocapsid to be transported into the nucleus where the HBV DNA is released and repaired (both the nick and the incomplete strand). This creates the stable progenome, known as cccDNA, covalently closed circular DNA from which transcription occurs (probably by human RNA polymerase). Several RNA species are transcribed, including small forms that serve as messenger RNA for production of HBsAg, HBcAg and DNA polymerase. In addition, a supra-genomic RNA is created that is the "pregenome", the RNA from which HBV DNA is reverse transcribed which is done inside of self-assembled HBcAg particles (with DNA polymerase enclosed). The polymerase synthesizes negative strand DNA and is then the pregenome is degraded (by the multifunctional polymerase). The positive HBV DNA strand is then synthesized but is not completed. Somehow the presence of double stranded DNA in the core particle signals the addition of HBsAg to the particle which is then exported into the serum. Understanding the details of the HBV life cycle is important particularly for the development of therapies. Currently, all therapies are directed at DNA synthesis and inhibition of DNA polymerase which occurs within the core particle and thus is susceptible only to very small molecules like nucleoside analogues.

HBeAg and HBcAg Crystal Structures

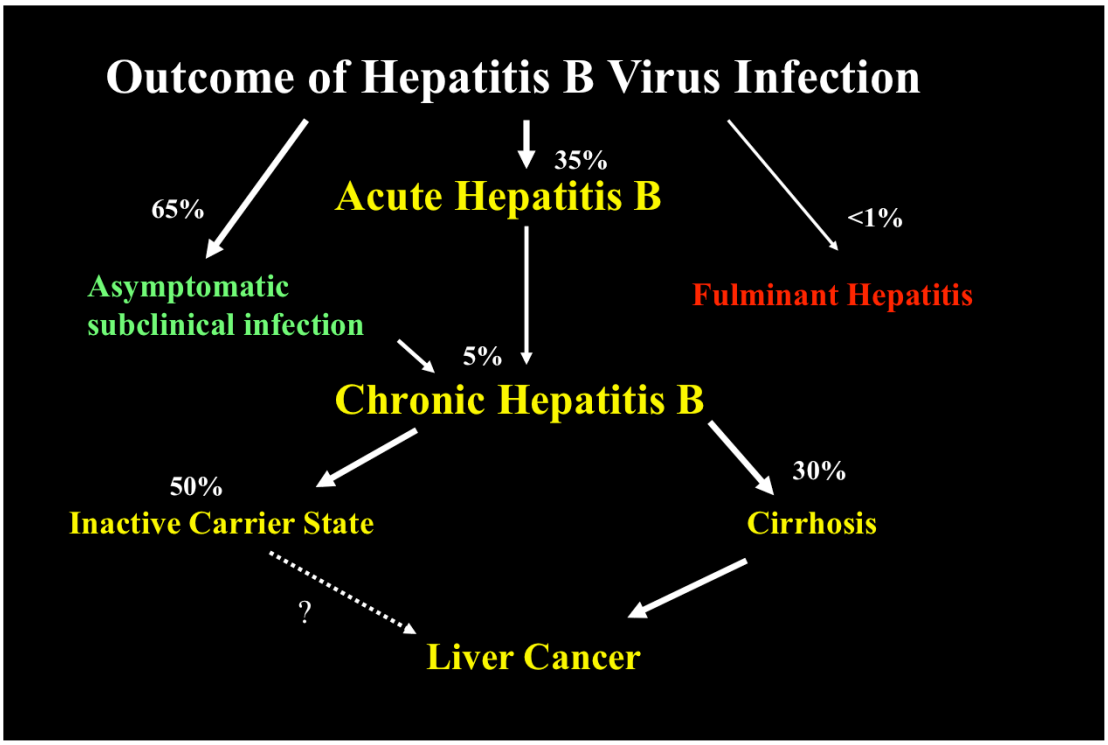


DiMattia et al. Structure 2013

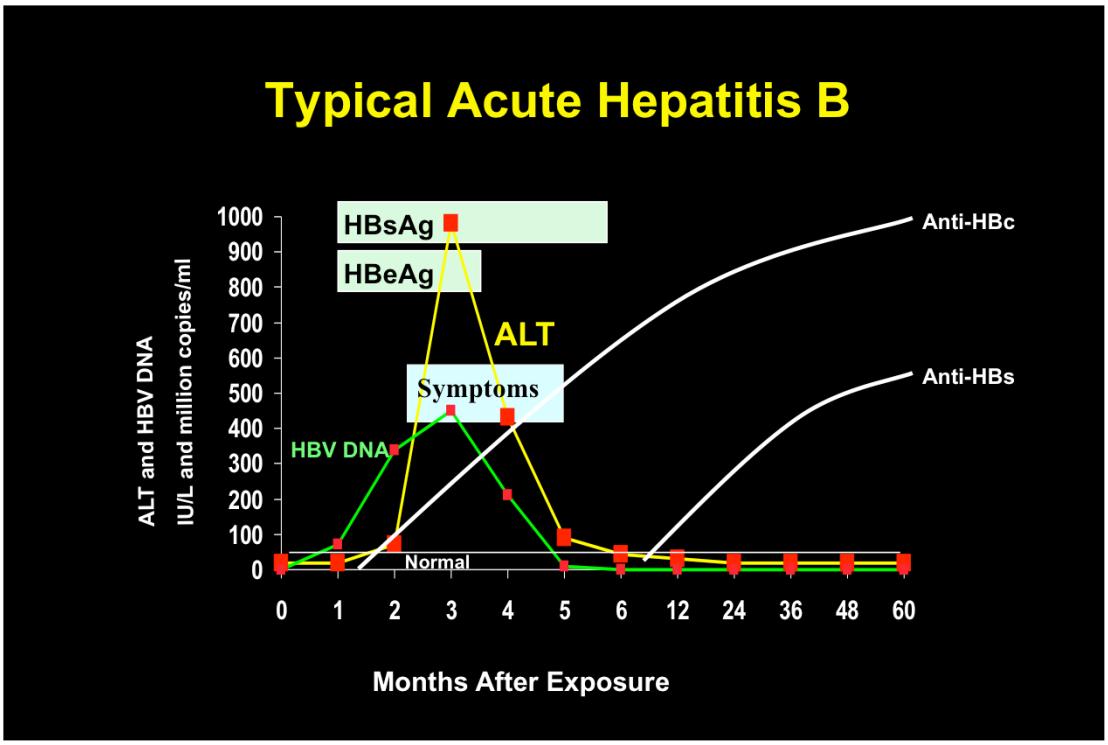
Recently, X-ray crystalographers were able to purify and analyze HBeAg and HBcAg demonstrating the different tertiary structures of these two molecules. A key component appears to be the S-S interaction which is possible because of the pre-code sequence in HBeAg and which results in its dimerization. Thus, T cell (which recognize short amino acid sequences) reactivity should be shared between HBeAg and HBcAg; but B cell (which recognize tertiary structure) reactivity is not (thus anti-HBe and anti-HBc are different). Circulating HBeAg is, therefore, not neutralized by anti-HBc, but can interact with circulating HBcAg specific T cells. This suggests that HBeAg is a immunomodulatory antigen that promotes chronicity by blocking T cell responses to HBV core antigen. This hypothesis fits all of the clinical features of hepatitis B and the association of HBeAg with high levels of viral replication and with evolution to chronic infection.



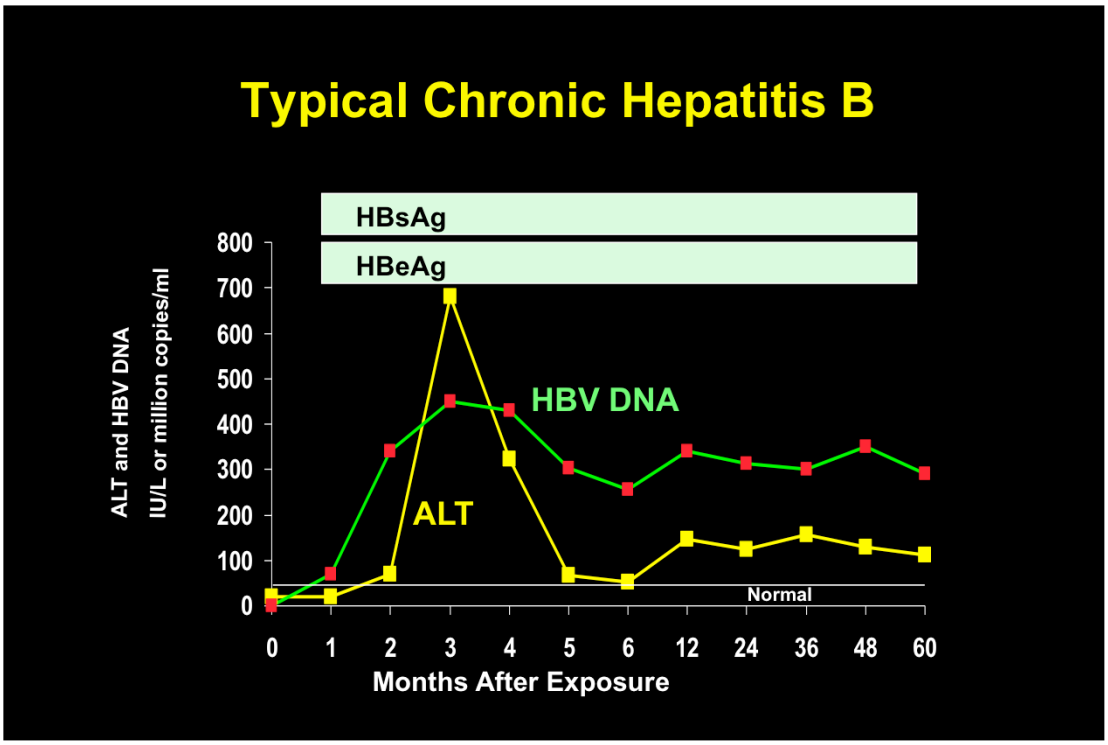
Mutations in the pre-core region can result in loss of HBeAg. Once chronicity is established, HBeAg appears to help with keeping viral replication high, but is not necessary for perpetuation of chronicity. Once HBeAg is lost, levels of virus decline (sometimes to undetectable) and the clinical disease usually improves. Some patients, however, continue to have active disease: called “HBeAg-negative chronic hepatitis B”.



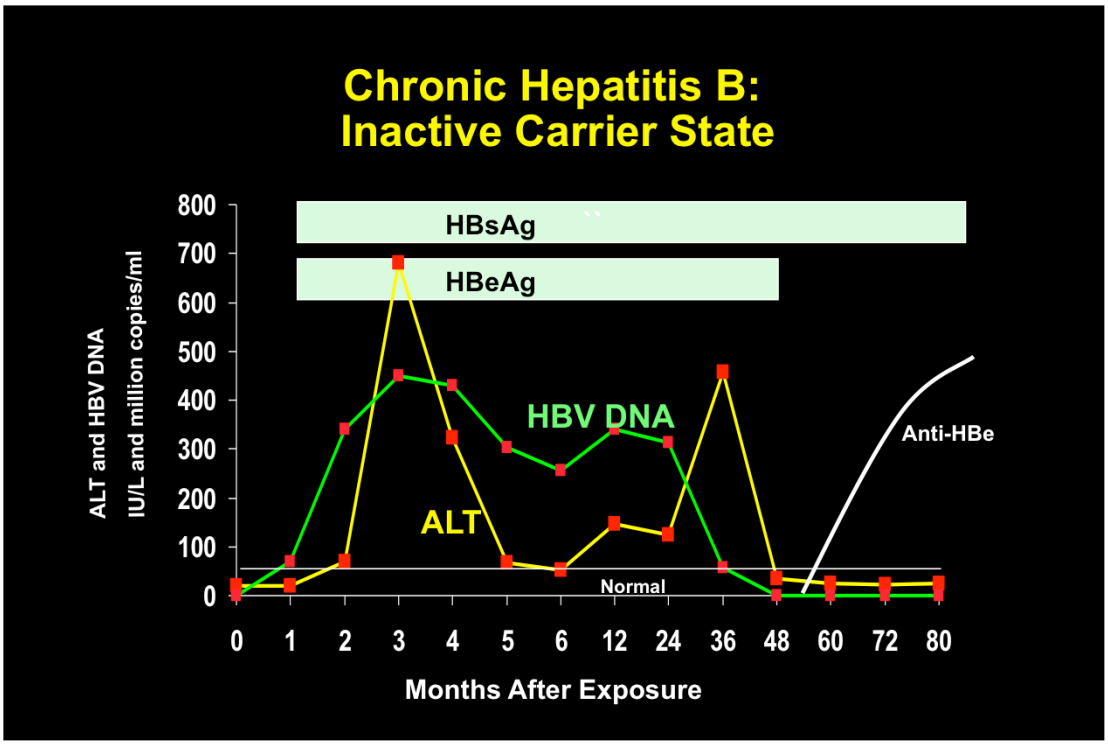
In adults, about 35% of patients develop clinically apparent acute hepatitis with jaundice. A small proportion of these have fulminant hepatitis which can be fatal. Most adults, however, have a subclinical infection and never know that they have had hepatitis B. About 5% of these subclinical infections, however, result in a chronic infection. Thus, patients found to have chronic hepatitis B usually deny a history of acute hepatitis. The disease is often "silent" and is not manifest until cirrhosis or liver cancer arises years or decades later. Also a high percentage of persons with chronic hepatitis B ultimately have a remission in disease, loss of HBeAg and become "inactive carriers" with HBsAg in serum but no evidence of active disease and no elevations in liver enzymes such as ALT (alanine aminotransferase) or AST (aspartate aminotransferase).



This is the typical serologic course of acute hepatitis B. HBV DNA arises during the incubation period and peaks at the time of clinical onset (ALT elevation), disappearing with recovery. HBsAg and HBeAg also appear and are then cleared whereupon anti-HBe (not shown) and anti-HBs arise. Anti-HBc arises at the time of onset and is the best serological marker of HBV infection (as opposed to immunization, whereafter only anti-HBs is produced).



The serology of chronic hepatitis B differs from acute, resolving disease in the persistence of HBsAg, HBeAg and HBV DNA in serum. The clinical disease is usually mild and asymptomatic, but it occurs. With chronic hepatitis B, the liver enzymes are usually only mildly or moderately elevated and the patient may have no symptoms or only vague symptoms of fatigue and slight ache over the liver.

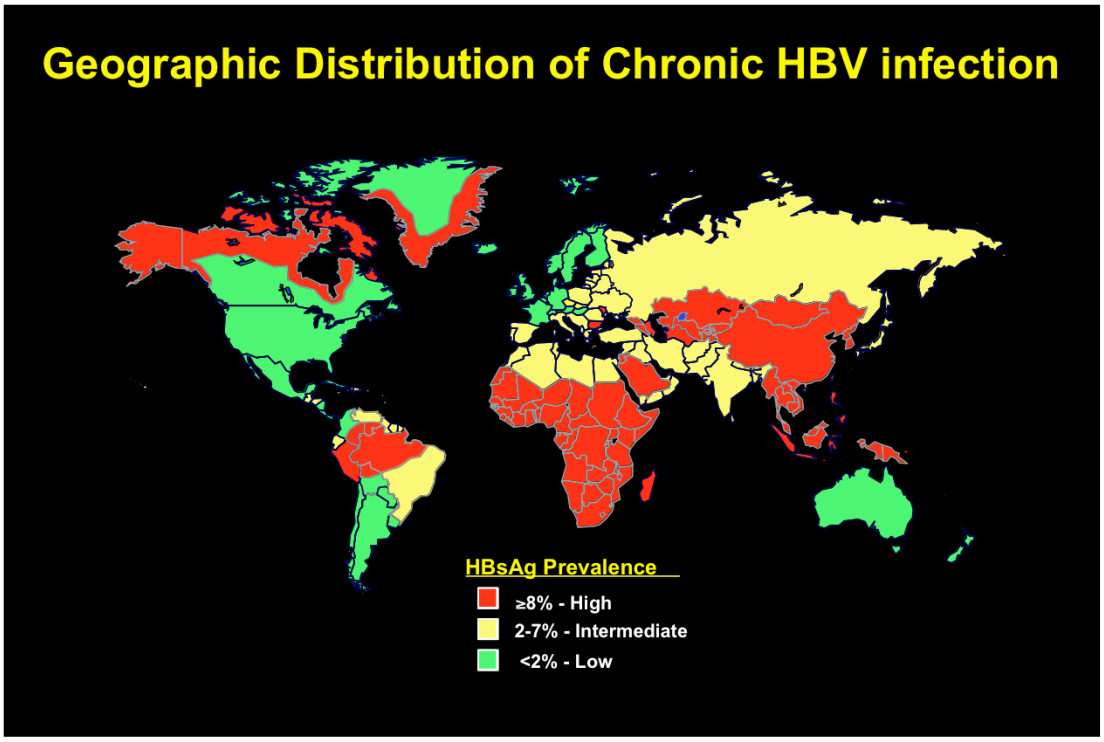


In many patients with chronic hepatitis B, the disease resolves, usually have a minor flare and with clearance of HBeAg and development of anti-HBe which is followed by liver enzymes falling to normal. HBsAg persists. It is not clear what continues the production of HBsAg, but it appears to be defective molecules of cccDNA in liver cells which produce HBsAg efficiently, but produce only low levels of viable intact virions. Thus, these patients have HBV DNA in liver, but little or none in serum.

Hepatitis B Virus Mutants

- Variations in nucleotide sequence in one of the HBV genes can result in change in the virological and, in some cases, clinical features of the infection.
- S gene: vaccine or HBIG escape mutants
- C gene: can affect disease severity or serological and clinical manifestations
- P gene: can effect replicative efficiency and resistance to antiviral therapy

HBV DNA can mutate and mutations have different clinical outcomes.

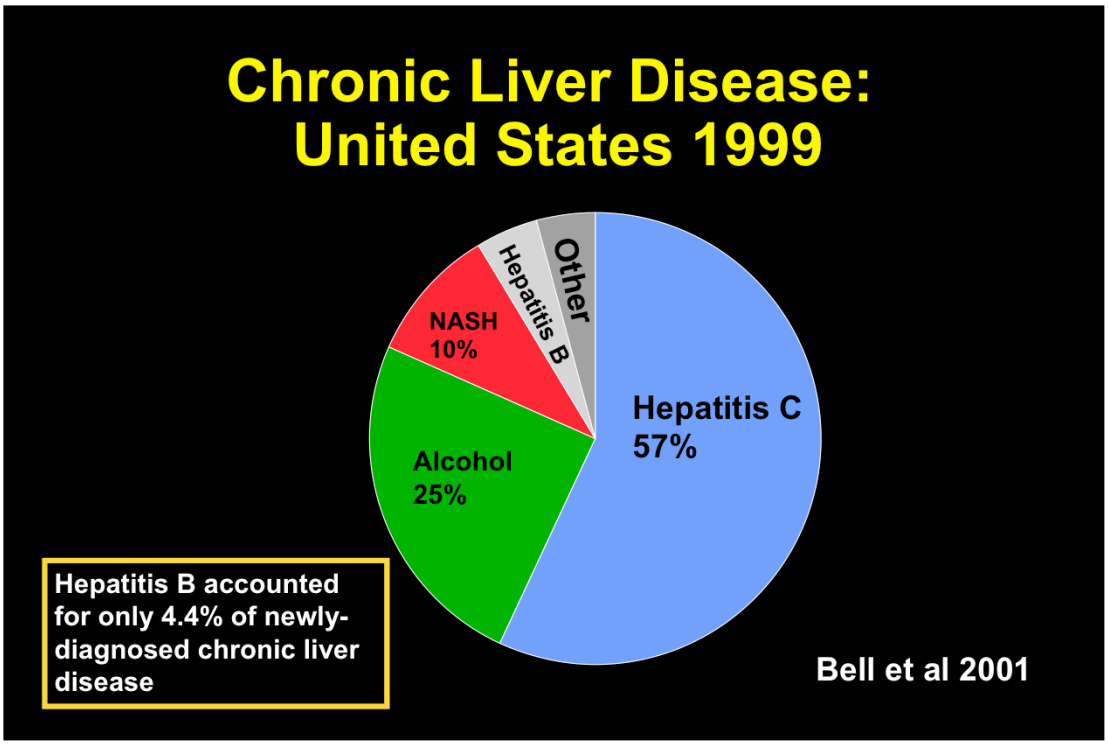


The prevalence of HBsAg varies greatly throughout the world, being common in Southeast Asia, China and Subsahara Africa and uncommon (usually <1% of the population) in the developed world such as the US, Europe and Australia.

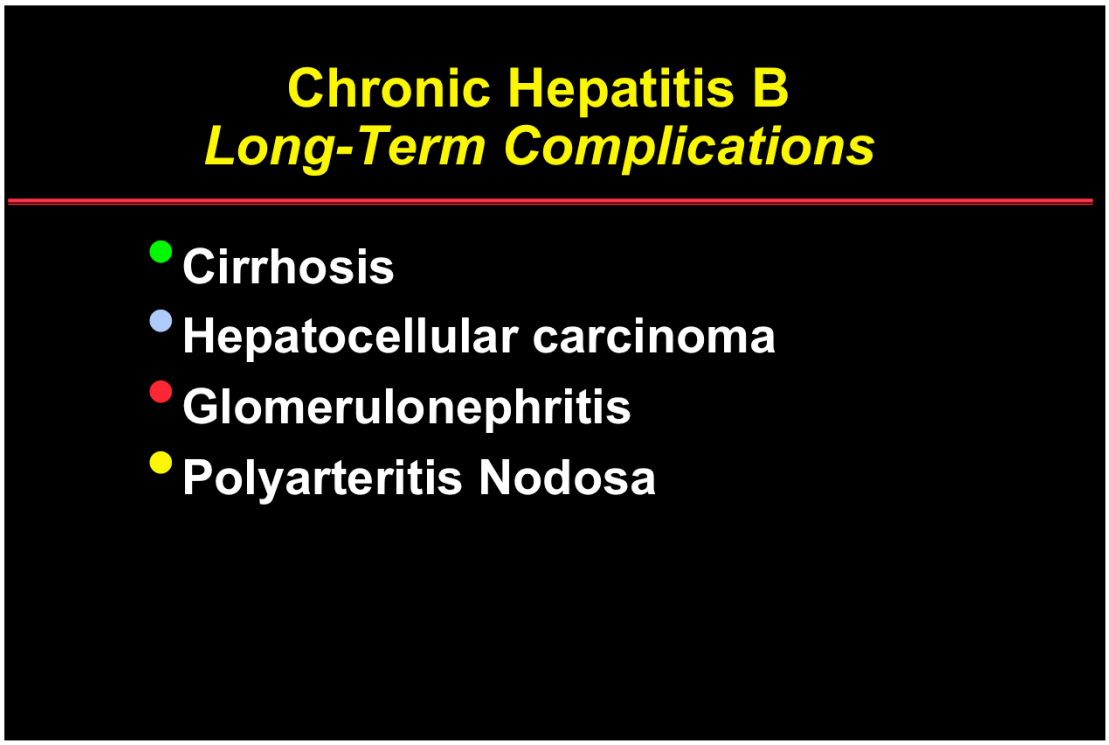
Genotypes of Hepatitis B Virus

Type	Subtype	Geographical Distribution
A	*adw*, *adw2*, *ayw1*	US, Northern Europe, Africa
B	*adw2*, *ayw1*	China, Indonesia, Vietnam
C	adr, *ayr*	China, Korea, Japan, Vietnam
D	*ayw2*, *ayw3*	Mediterranian, Middle East, India
E	*ayw4*	West Africa
F	*adw4*	Polynesia, Central and South America
G		Europe, US (rare)

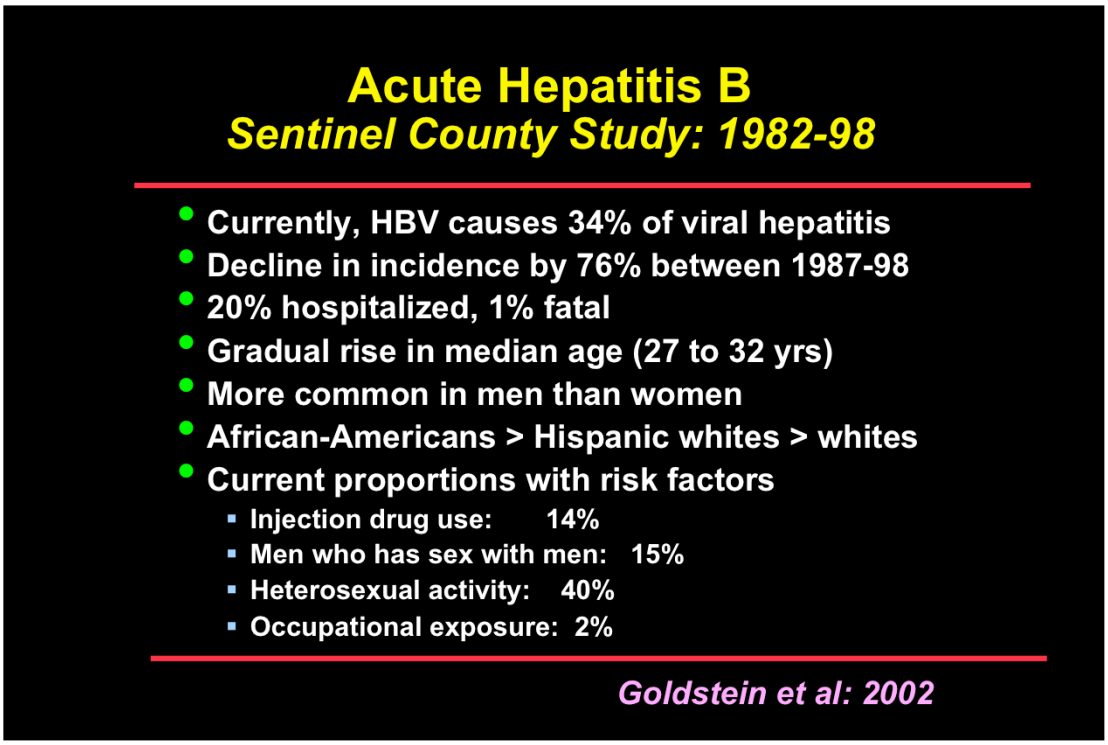
Different strains or genotypes of HBV have been described, which also have distinct geographic variation and can be used to map population migrations in history. In the U.S., genotypes A and D are most common in Caucasians, while genotypes B and C are found in Asian-Americans. There are a few clinical differences associated with genotypes but they are somewhat subtle. All genotypes can be associated with chronic hepatitis, cirrhosis and liver cancer.



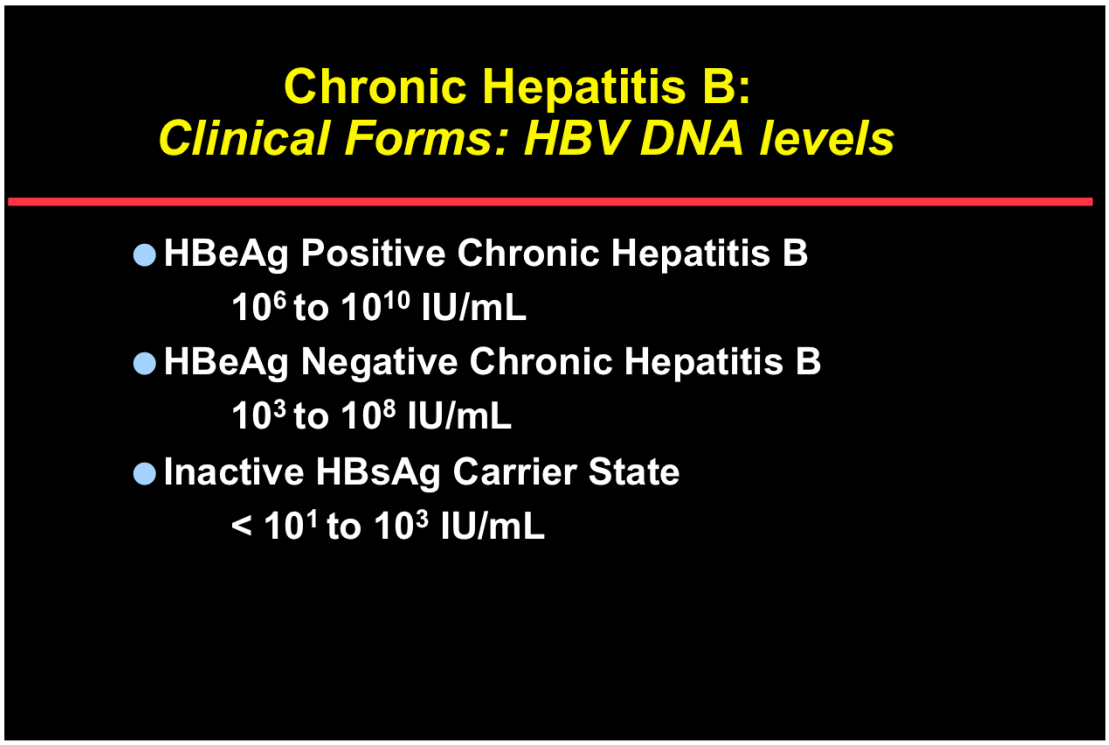
Hepatitis B is somewhat rare in the US. About 0.5% of the general population have HBsAg and hepatitis B represents about 4% of chronic liver disease, hepatitis C, alcohol and nonalcoholic fatty liver being more common. Furthermore, hepatitis B in the US occurs mostly in high risk groups: Asian-Americans, persons with a history of injection drug use or transfusion (before 1986) and persons with multiple sexual partners and particularly men-who-have-sex-with-men. These features are changing rapidly, because of the HBV vaccine.



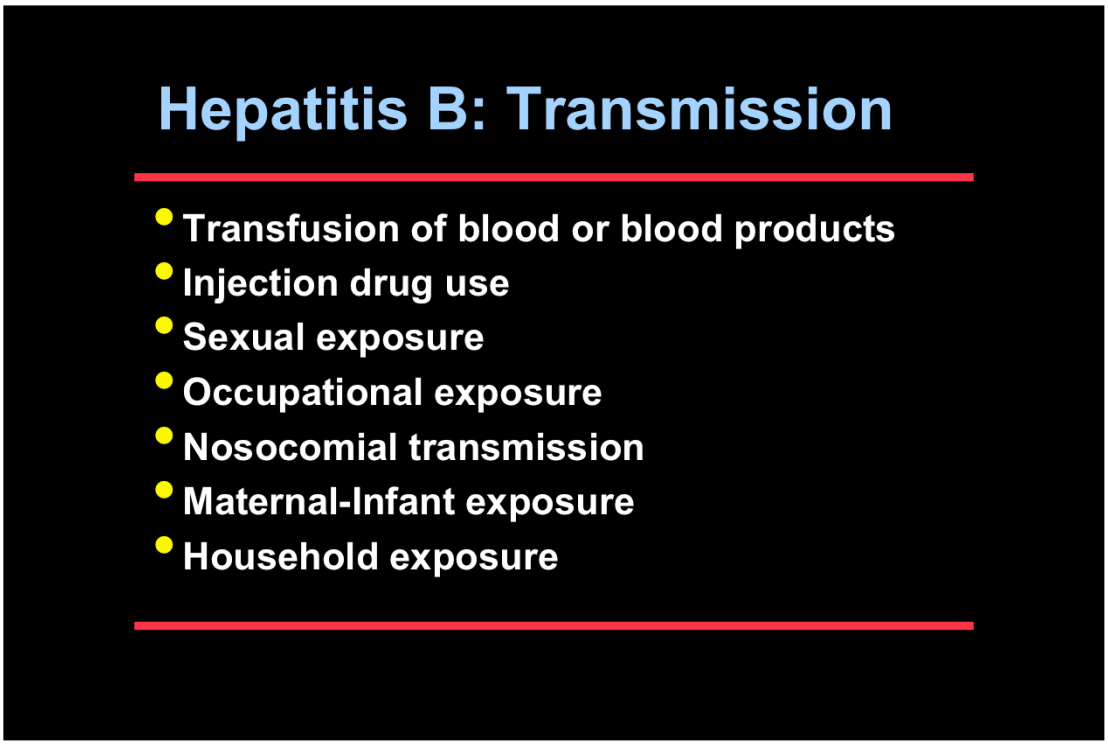
Worldwide, chronic hepatitis B is the most common cause of cirrhosis and liver cancer. These are long-term complications, usually arising after decades of infection.



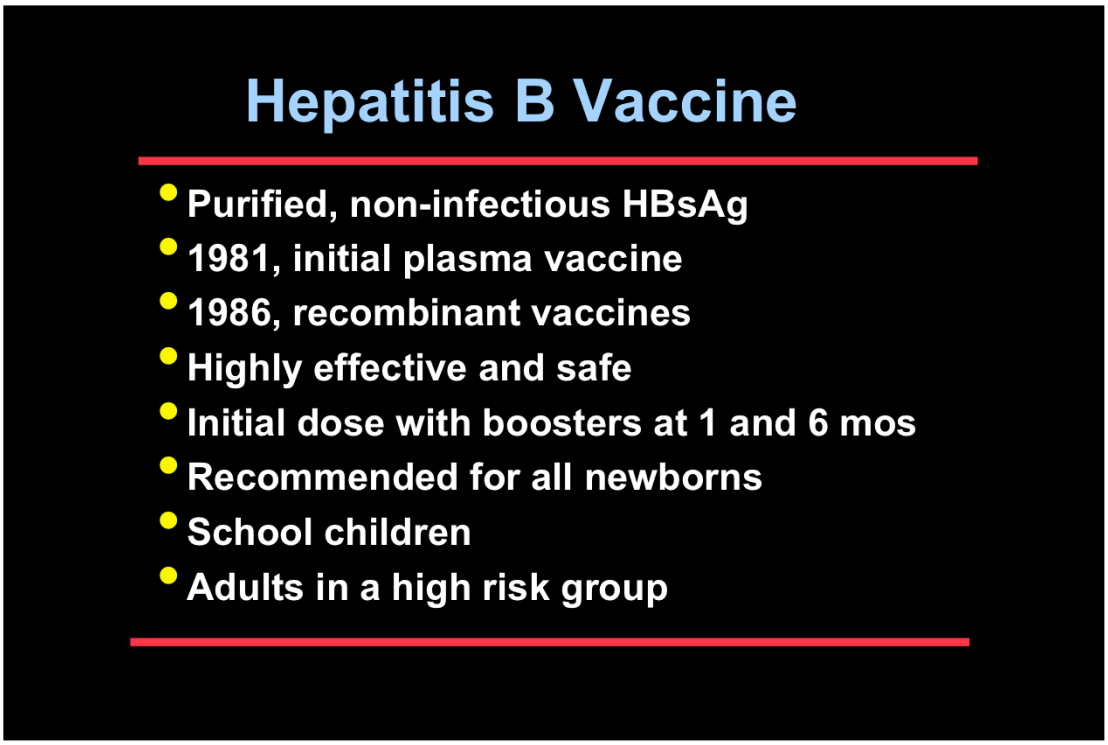
Risk factors in the US and generally due to high risk behavoirs or exposures that occur in adulthood. These features should drive recommendations for vaccination in these groups. Among Asian Americans, however, these risk factors are not very important as the disease is usually acquired in childhood, often from a mother who is an HBV carrier.



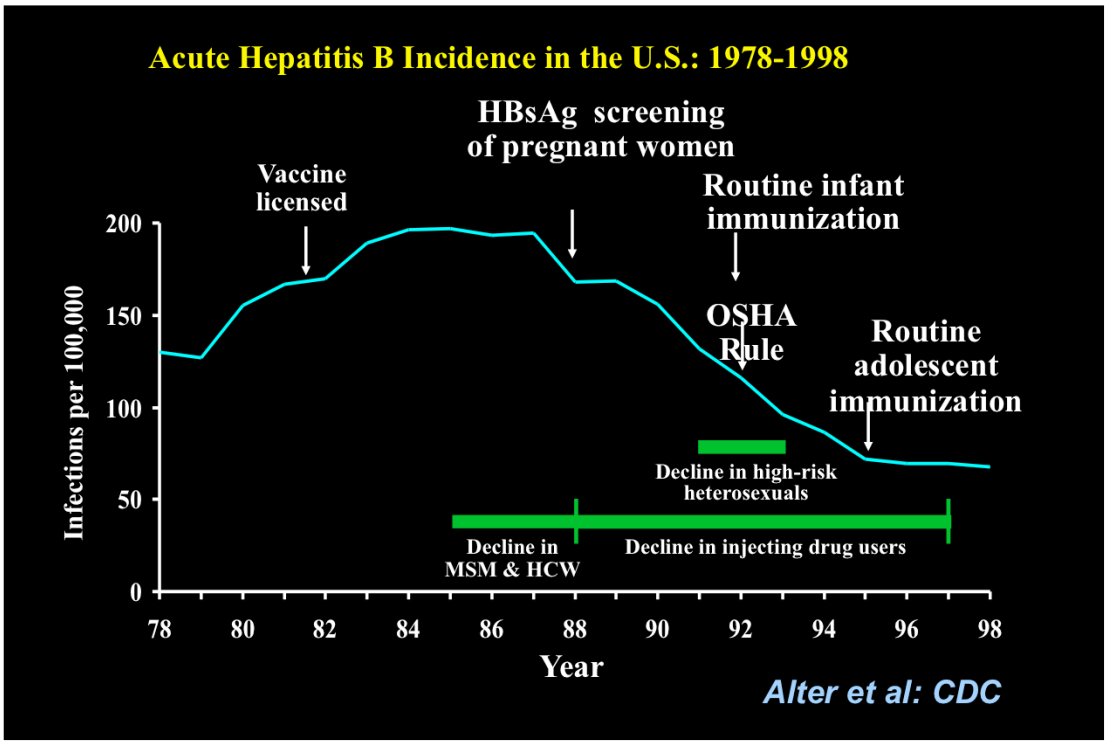
The three forms of chronic hepatitis B (this is a simplification, some patients cannot be clearly classified into one of these groups).



Risk factors for hepatitis B



A major accomplishment of the 20th century: a safe and effective HBV vaccine. The first recombinant vaccine for general use in humans. Now recommended for all newborns and for adults in high risk groups. With this accomplishment, cases of acute hepatitis B represent a failure of the medical care delivery system.



The effects of various public health measures which have resulted in acute hepatitis B being a rare disease, fewer than 2000 cases being reported in the US each year, a greater than 90% drop in incidence. [This slide is a bit dated]. Why should this not be a 100% drop?

Hepatitis B Transmission

- Hepatitis B is a preventable disease !
- Development of HBV vaccine was one of the great achievements of medical research in the 20th century
- Introduced in 1982, HBV vaccination has already been shown to decrease the rate of hepatocellular carcinoma
- Why do we continue to have new cases of acute and chronic hepatitis B in the U.S.?

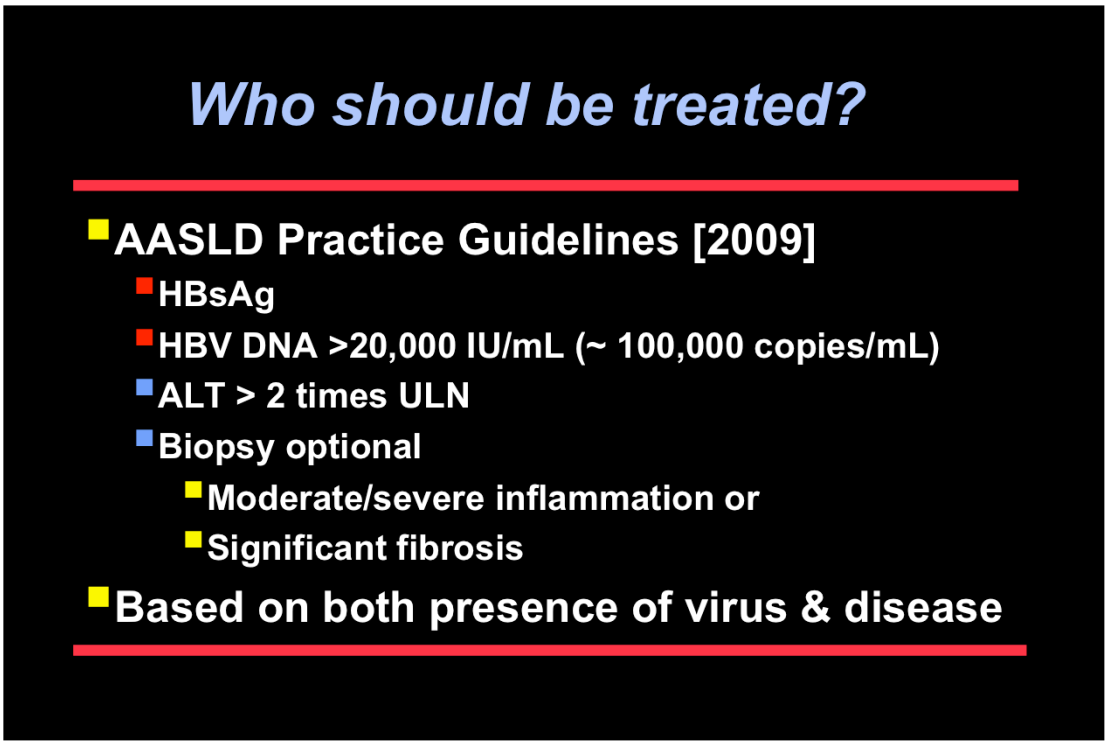
Who is this?

Sun Yat-sen (1866-1925)

The father of modern China, Sun Yat-sen (also a physician!). He died in 1925 at the age of 58 (young for an Asian man). The cause was not warfare or assassination, but hepatocellular carcinoma. Consider how hepatitis B has affected world history. He had managed to keep the forces of Chinese rebellion together, his supporters included Chang and Mao. Had Sun Yat-sen lived to be 80 or 90, the history of modern China might have been far different! The importance of hepatitis B in China led those nations to be the first to recommend universal HBV vaccination, first accomplished in Taiwan.

Therapy of Hepatitis B

Recently, hepatitis B has become a treatable disease

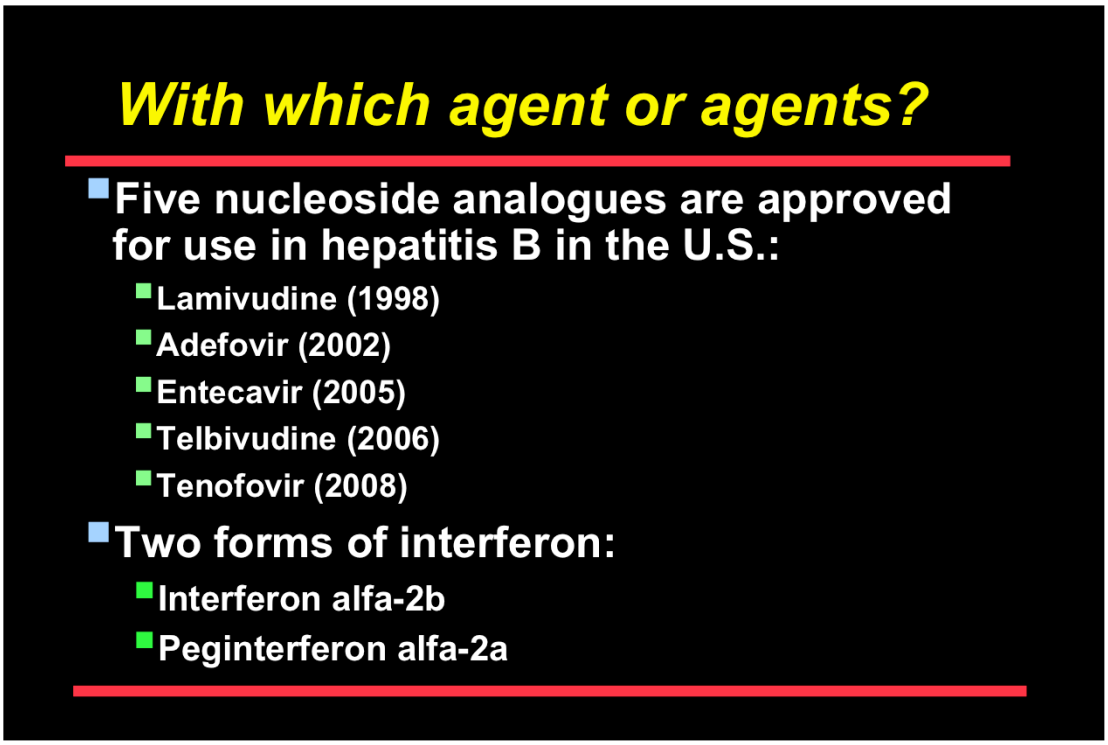


Who should be treated. Those with HBsAg but also the presence of virus (HBV DNA above a cutoff) and disease (ALT elevations).

Chronic Hepatitis B: *Three Clinical Forms:*

- **HBeAg Positive Chronic Hepatitis B**
  - ***HBeAg, raised ALT, HBV DNA in serum and chronic hepatitis on biopsy***
- **HBeAg Negative Chronic Hepatitis B**
  - ***Anti-HBe, raised ALT and HBV DNA in serum, chronic hepatitis on biopsy***
- **Inactive HBsAg Carrier State**
  - ***Anti-HBe, normal ALT & no HBV DNA, minimal nonspecific changes on biopsy***

Treatment is recommended for the first two categories of chronic HBV infection, but not for "inactive" carriers. Sometimes, it is difficult to separate categories 2 and 3; patients need to be followed and decisions based upon more than one determination.

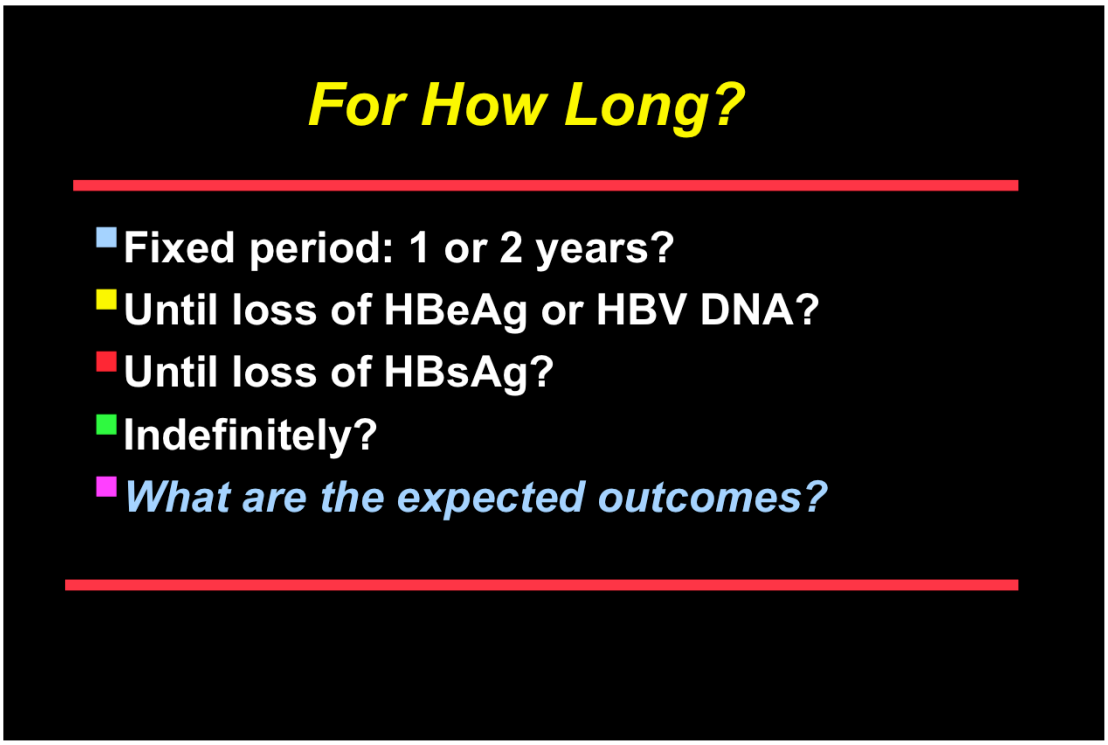


The nucleoside analogues with activity against hepatitis B have largely replaced interferon therapy which is difficult, limited in application by contraindications, and effective in only 30% of persons.

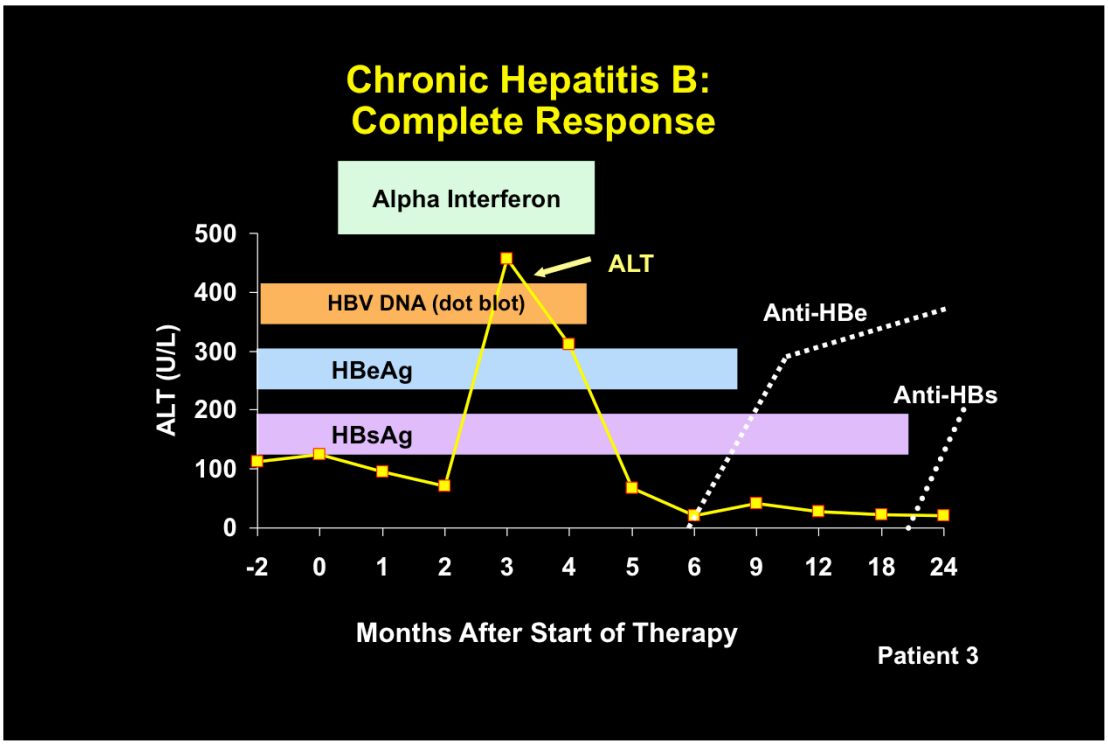
With which agent or agents?

- Three classes of nucleoside analogues
  - L-nucleosides (lamivudine, telbivudine)
  - Acyclic nucleotides (adefovir, tenofovir)
  - Cyclopentyl guanosine analogue (entecavir)
- All have similar general mechanism of action: HBV DNA polymerase inhibitors:
  - L-nucleosides: high cumulate rates of resistance
  - Acyclics: potential for renal toxicity
  - Entecavir: limited efficacy in patients with lamivudine resistance

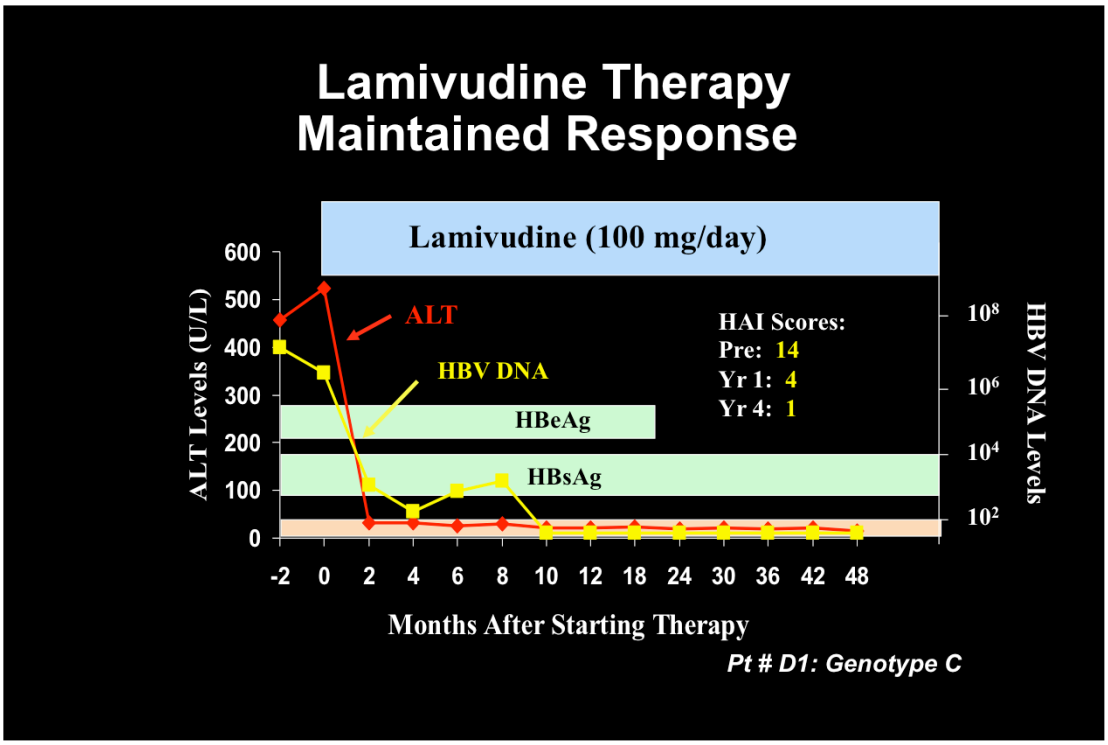
Importantly, the initial nucleoside analogues were associated with a high rate of acquired antiviral resistance (rising to greater than 50% after several years of therapy). The most potent agents are tenofovir and entecavir, both of which have a very low rate of resistance (<1% after 4-5 years except in persons with pre-existing resistance from the other agents). Currently, these two agents are recommended.



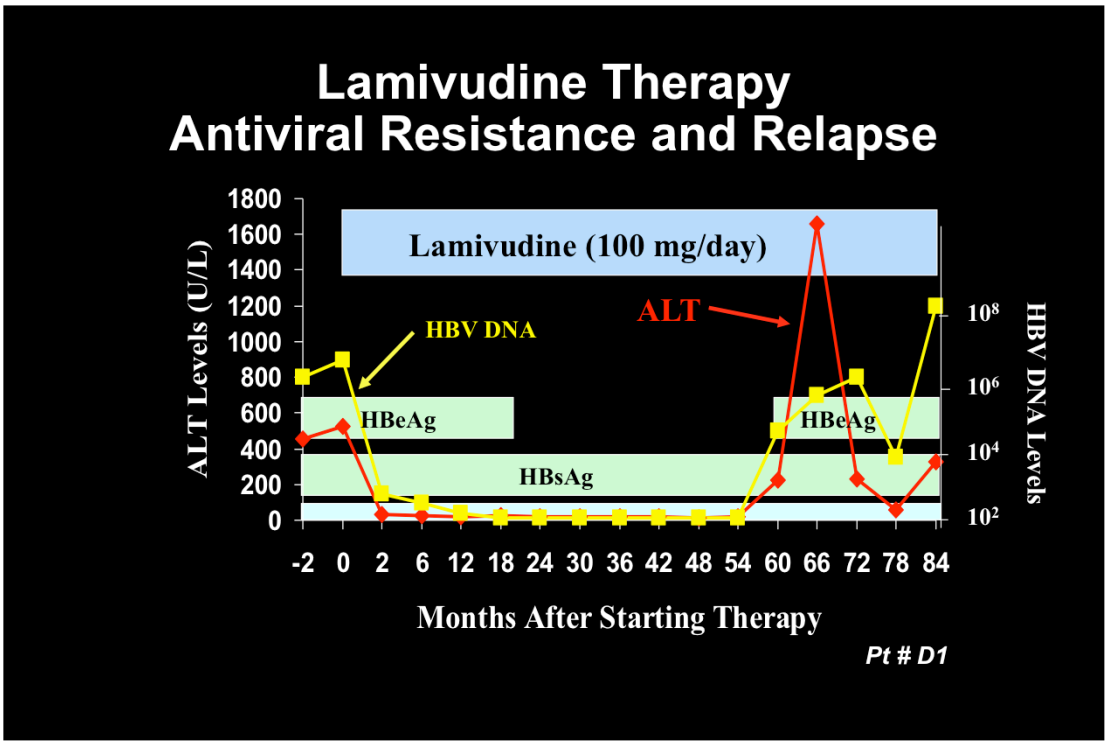
Therapy, however, usually does not “cure” hepatiis B or result in loss of HBsAg. Should these agents be used long-term (like antiretroviral agents) or should they be given as a prolonged course (such as for tuberculosis).



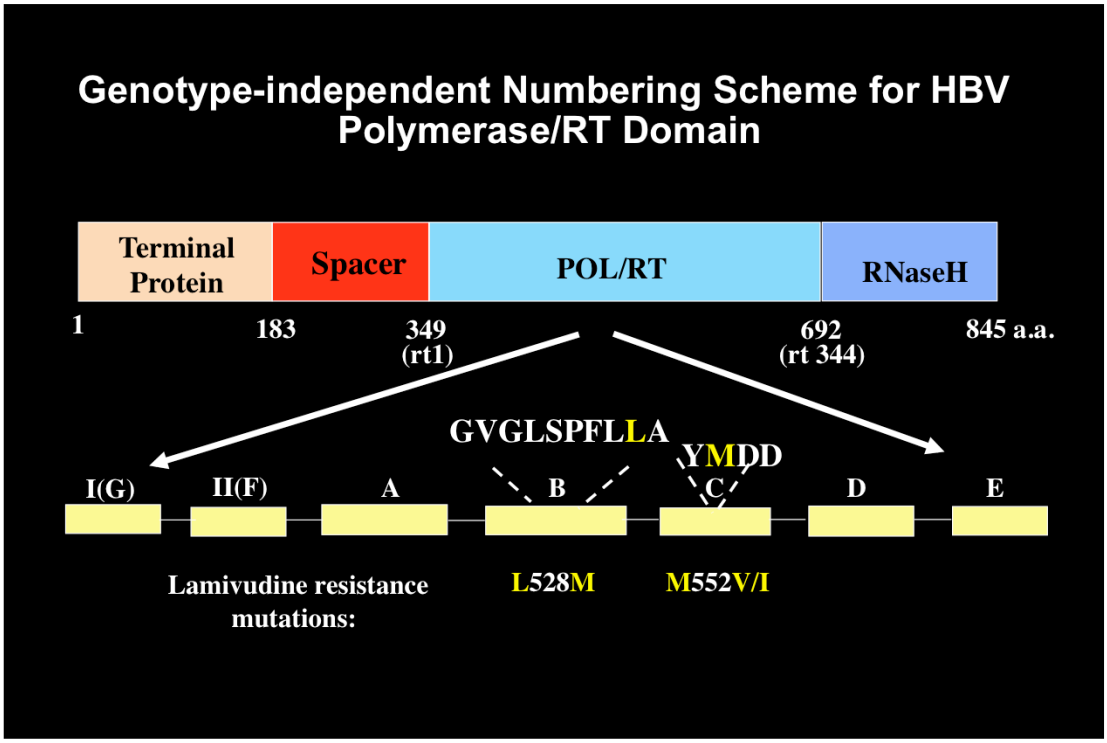
Interferon resulted in remissions in only 30% of selected patients. But the remissions were often excellent and long-term; not requiring continuous therapy. This patient lost HBV DNA during therapy and later HBeAg followed (2 years later) by loss of HBsAg and anti-HBs.



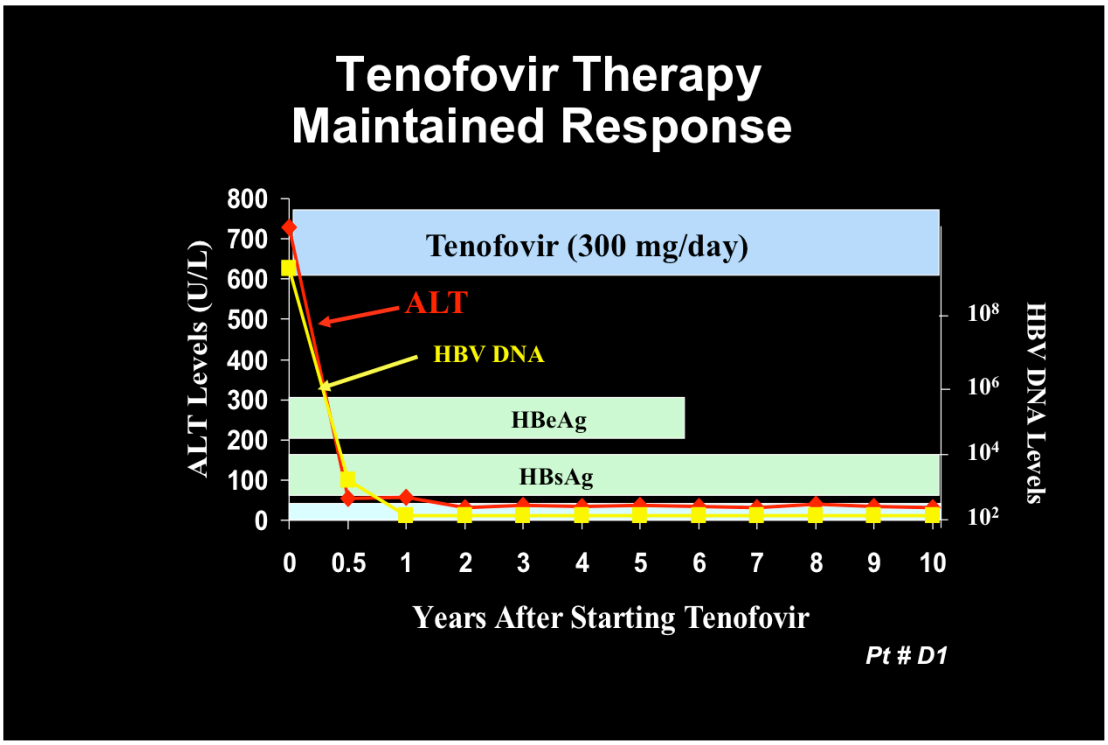
Lamivudine and other nucleosides, however, are effective in almost 100% of patients, lowering levels of HBV DNA within months and often resulting in marked improvement in the liver disease and long-term suppression of HBV DNA to undetectable levels. This person became HBeAg negative but remained HBsAg positive. Chronic hepatitis B, however, is a chronic disease and even four years does not represent it adequately. The next slide shows his subsequent course.



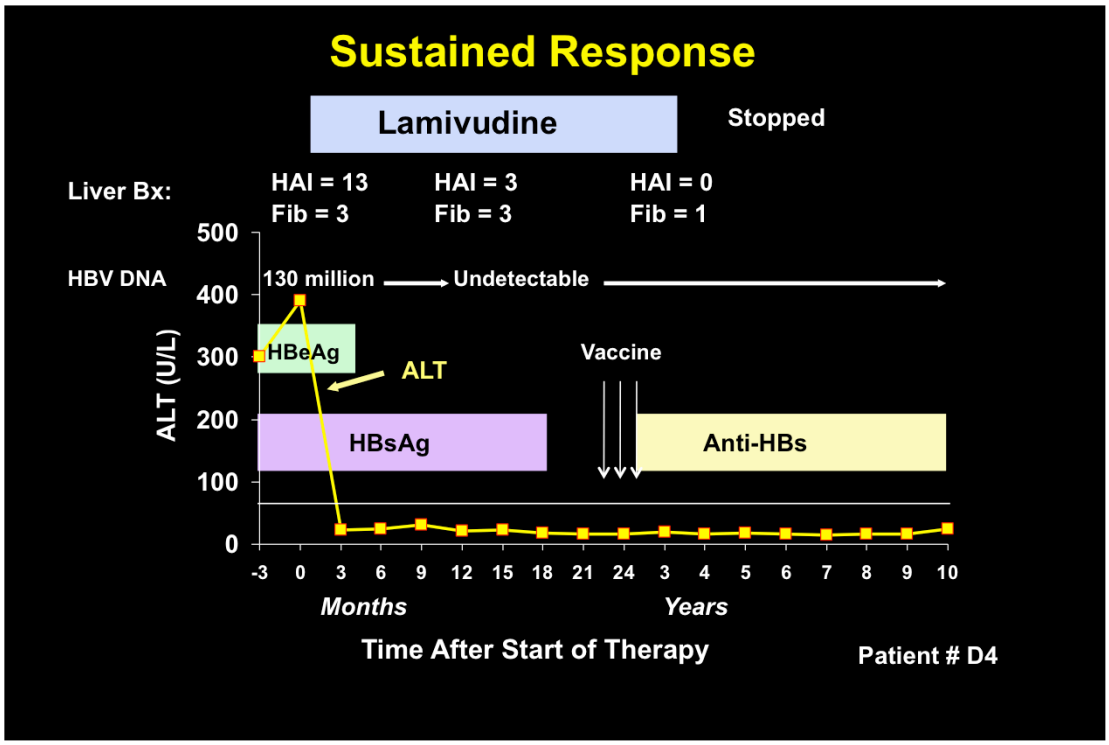
A year after a 4-year biopsy showing resolution of the liver injury, HBV DNA became detectable followed by HBeAg and a flare of hepatitis. He had developed antiviral resistance to lamivudine and molecular sequencing showed the presence of a characteristic mutation in the polymerase gene associated with a change in the catalytic region of the enzyme that blocks its ability to inhibit the enzyme



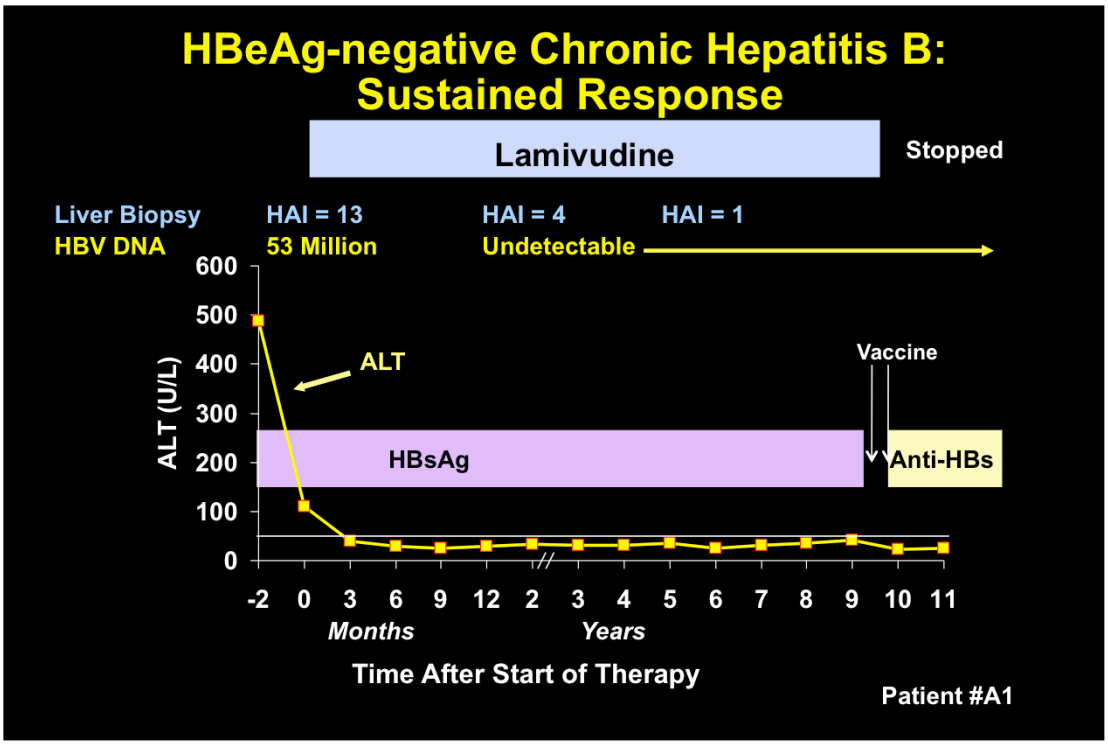
Mutation in the “C” region of the polymerase gene from M to either V or I is the typical finding in patients with lamivudine resistance and arises in 25% of patients each year, peaking at 70-75% after 4 to 5 years.



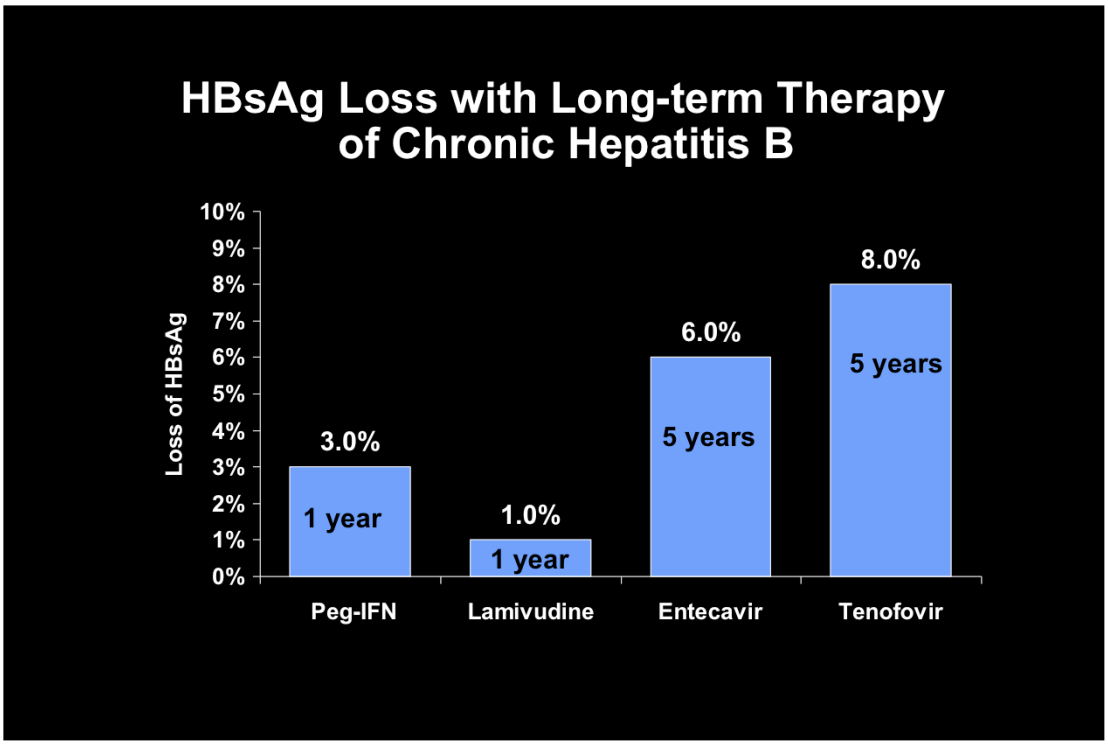
This patient was then placed on tenofovir and again had a dramatic reduction in HBV DNA (to undetectable levels within 1 year, followed by improvements in ALT. With this course, it took almost 6 years of therapy for the loss of HBeAg and he remains HBsAg positive 10 years later (he faithfully takes tenofovir, once daily). Can it be stopped? [he is not willing to stop it!]



Lamivudine can be successful, in about 30% of patients (those who do not develop resistance). He is an example of someone who lost HBeAg after 6 months and because HBsAg negative after 18 months. He did not develop anti-HBs, so a course of HBV vaccine was given. When he developed anti-HBs, lamivudine was withdrawn. He remains with normal ALT levels, no detectable HBV DNA or HBsAg, 7 years later. This is the best we have as an example of "cure". But is it really? The answer is no; this is a long-term remission, but the disease can come back in what is called "reactivation". Reactivation is typical of DNA virus infections in that the viral genome remains in the body and can be reactivated to replicate with immune suppression such as with cancer chemotherapy or immunodeficiency from AIDS infection. Patients such as this should be informed and warned about this possibility. If cancer chemotherapy is needed in the future (or immunosuppression for a conditions such as asthma, rheumatoid arthritis, etc), anti-HBV therapy should be restarted.



Another example of loss of HBsAg after long term maintained suppression of HBV.



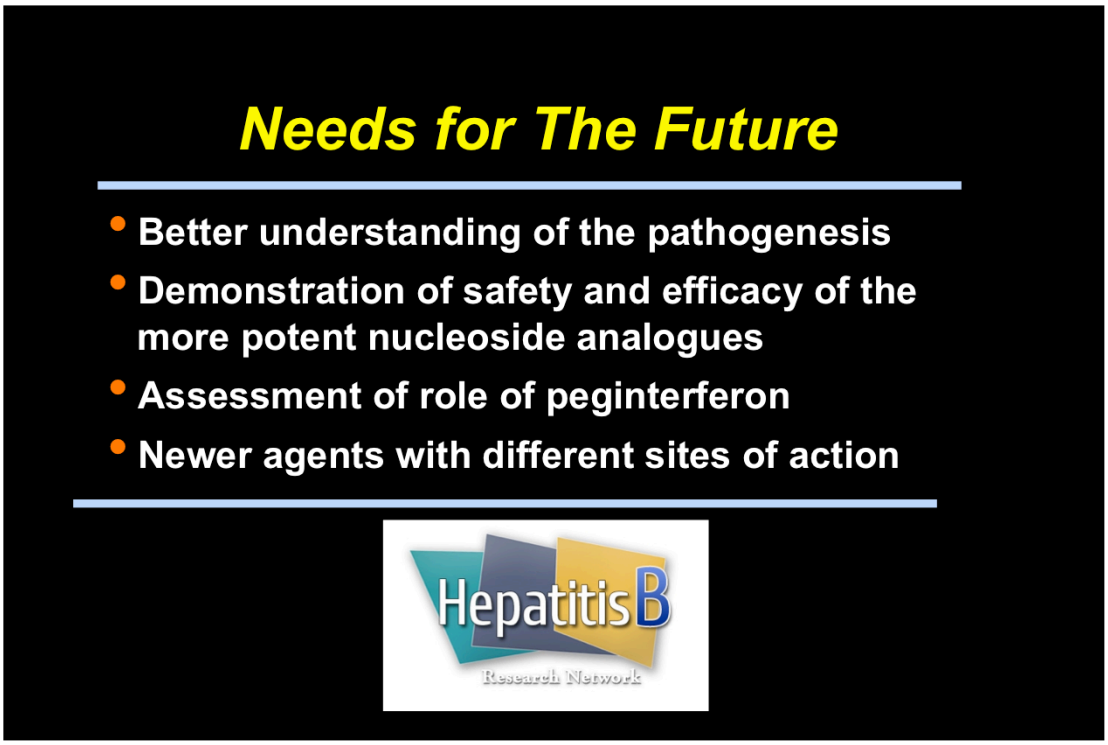
Here are estimates of the rate of loss of HBsAg with long-term use. It seems to rise by ~2% per year. These findings indicate that we have excellent therapies for hepatitis B but need something more – that can help with the loss of HBsAg. This is the major challenge in HBV research today.

Therapy is excellent but there are shortcomings. Actually few people question the long-term benefit of therapy and the demonstration of improvement in survival is just a matter of time.

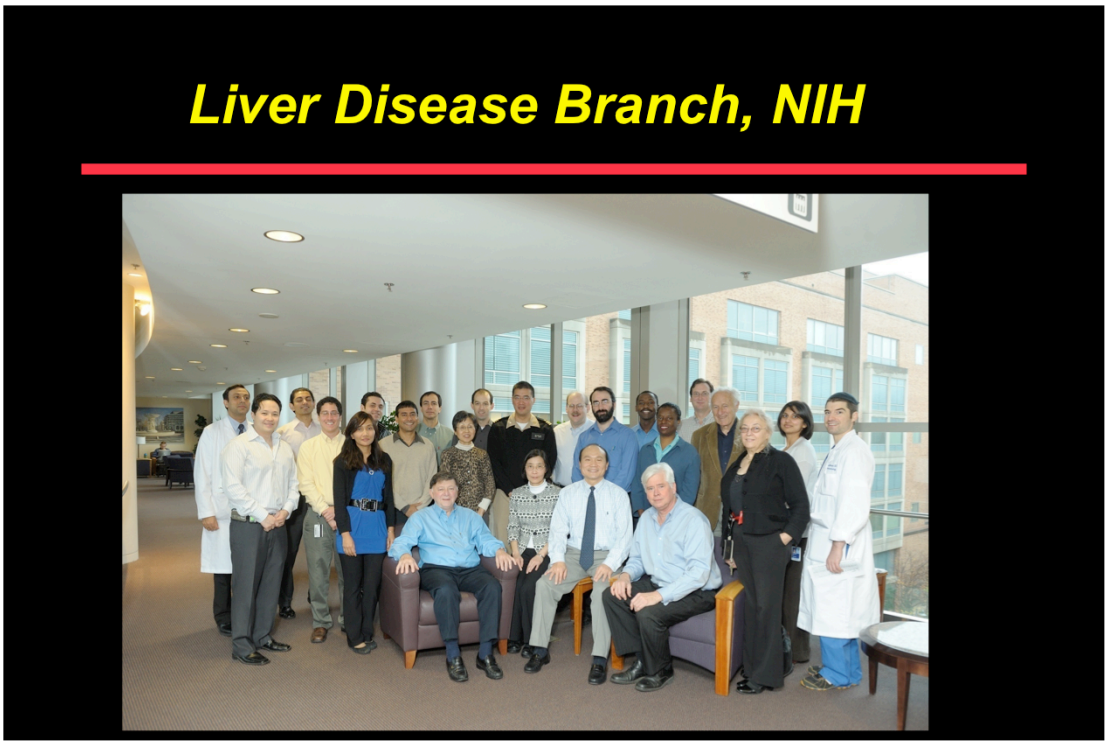
Costs of Therapy of Hepatitis B

Drug	Dose	~Cost/Year
Lamivudine	100 mg	$3,445
Telbivudine	300 mg	$6,848
Adefovir	10 mg	$7,832
Tenofovir	300 mg	$5,560
Entecavir	0.5 mg	$8,297
Peginterferon	180 mcg	$20,222

Costs are high as is typical for all new medications being developed. Tenofovir and entecavir are likely to come “off patent” in the next 2-3 years and the price is likely to drop (this is particularly important for those areas of the world where hepatitis B is most common and has greatest effect on morbidity and mortality).



The HBRN was developed as a prospective study of hepatitis B in the US with multiple aims, focusing upon improving management and outcome of this disease.



The Liver Diseases Branch, NIDDK. The intramural group that conducts basic and clinical research on HBV, led by Dr. Jake Liang (front row sitting with tie). This photo shows the clinical team with several honored visitors including Win Arias (standing, 4th from right), Anna Lok (sitting 3rd from right), and Vic Navarro (last row standing 4th from left).